INTRODUCTION

Annona reticulata Linn. (Annonaceae), commonly known as bullock’s heart, is widely distributed in India; they are tall, with many branches, bearing nutritious fruits. Plants belonging to genus Annona such as Annona muricata Linn. (Annonaceae), Annona glabra Linn. (Annonaceae), and Annona cherimola Mill. (Annonaceae) have been reported to be used as hypoglycemic agents in Mexico [1]. Several species of the genus Annona such as Annona squamosa Linn. (Annonaceae) [2], Annona cherimola Mill. (Annonaceae) [3], A. muricata Linn. (Annonaceae) [4,5], Annona macrophyllata Donn. Sm. (Annonaceae) [6] had been reported for their anti-diabetic effect. The taxonomic profile of the plant and unavailability of sufficient scientific reports regarding the hypoglycemic potential of A. reticulata makes it an interesting plant for investigating the hypoglycemic potential of the plant if any. Our previous studies had revealed that the hydro-alcoholic extract of leaf of A. reticulata is having potential anti-hyperglycemic potential which is more corrective in nature [7] and made the plant more interesting to be investigated further. The phytochemical investigation by gas chromatography-mass spectrometry of different fractions made from hydro-alcoholic extract of leaf of A. reticulata revealed the presence of several compounds which may be responsible for the observed anti-hyperglycemic activity of the extract [8]. The focus of the present investigation had been to evaluate the efficiency of different fractions prepared from hydro-alcoholic extract of A. reticulata leaf in the regulation of blood glucose levels in streptozotocin (STZ) induced hyperglycemic rats.

METHODS

Plant collection, preparation of extract and its fractions

The flowering twigs of A. reticulata were collected in the month of July-August 2010, from the rural area of Cuttack District, Odisha, India, and authenticated by Dr. Kshetra Mohan Das, Central Rice Research Institute, Cuttack, Odisha, India, and a specimen voucher (Specimen No. 10-11/SPS/SAOU) was kept in the University for future reference. After identification and authentication of the plant, leaves of the plant were collected for the experimental process. The leaves were shade dried, made into coarse powder and the powdered material was initially defatted with petroleum ether and then subjected to cold maceration process for 72-h using 1:1 mixture of methanol and water as solvent to prepare hydro-alcoholic extract of A. reticulata leave (percentage yield 25.50% w/w with respect to dried powder). The extract was filtered and concentrated by rotary evaporator. For the preparation of different fractions method published by Sourav et al. (2012) was used [9]. The extract is mixed with water and methanol (1:1) and used for fractionation using chloroform, ethyl acetate, and methanol as solvents. After identification and authentication of the plant, leaves of the plant were collected for the experimental process. The leaves were shade dried, made into coarse powder and the powdered material was initially defatted with petroleum ether and then subjected to cold maceration process for 72-h using 1:1 mixture of methanol and water as solvent to prepare hydro-alcoholic extract of A. reticulata leaf (percentage yield 20.5% w/w with respect to dried powder). The extract was filtered and concentrated by rotary evaporator. For the preparation of different fractions method published by Sourav et al. (2012) was used [9]. The extract is mixed with water and methanol (1:1) and used for fractionation using chloroform, ethyl acetate, and methanol as solvents by the use of separating funnel. The different fractions prepared are chloroform fraction of hydro-alcoholic extract of A. reticulata (HAARC) (percentage yield 1.00% w/w with respect to concentrated extract), ethyl acetate fraction of hydro-alcoholic extract of A. reticulata (HAARE) (percentage yield 15.50% w/w with respect to concentrated extract), methanolic fraction of hydro-alcoholic extract of A. reticulata (HAARM) (percentage yield 12.80% w/w with respect to concentrated extract), and residual fraction of hydro-alcoholic extract of A. reticulata (HAARR) (Percentage yield 25.50% w/w with respect to concentrated extract). HAARC was not used for the experimental purpose as the yield volume of the fraction was very less, whereas all other fractions were used for...
determination of their anti-hyperglycemic activity. The extract and all the fractions were stored at room temperature in desiccators till further use.

**Preparation of interventions**

The fractions and the hydro-alcoholic extracts were suspended in distilled water using 25% Tween 20 as suspending agent for the preparation of test interventions. The suspension of standard drug metformin was also prepared in a similar manner. The dose at which the test substances were administered was 100 mg/kg, and the standard drug was administered as a dose level of 300 mg/kg. Suspension of distilled water and 25% Tween 20 was used as solvent treatment throughout the study at a dose of 2 ml/kg. The solvent, test samples, and standard drugs were administered by oral route based on dose and corresponding weight of the animals.

**Experimental animals**

Healthy Wistar albino rats (180-250 g body weight) supplied by Central Animal House of School of Pharmaceutical Sciences, SOA University, Bhubaneswar, India, were used in the experiments. The animals were acclimatized to laboratory conditions for 1 week before commencement of the experiment. The study was approved by University Animals Ethics Committee (Regd. No. 1171/C/08/CPSSEA).

**Induction of diabetes**

Diabetes was induced as per the method published by Sachin et al. (2009) [10]. Hyperglycemia was induced by intraperitoneal administration of multiple low doses (40 mg/kg) of STZ to the overnight fasted rats for 5 consecutive days. STZ solution was prepared freshly in ice-cold citrate buffer (0.01 M, pH 4.5). After 12 days of STZ administration, the fasting blood glucose (FBG) levels were measured, and the rats with FBG level ≥250 mg/dL were considered as diabetic and used for experimental purpose. Blood glucose level was estimated by using Glucometer (One Touch Horizon, Lifescan, Johnson and Johnson Company).

**Acute toxicity study**

Healthy Wistar albino rats of either sex starved overnight were divided into five groups (n=4). Groups I-III animals were orally fed with different fractions with increasing dose levels of 0.25, 0.5, 0.75, and 1.0 g/kg, body weight; Animals of Group IV were treated with the extract with increasing dose levels of 0.25, 0.5, 0.75, and 1.0 g/kg body weight, while Group V (untreated) served as control. The animals were observed continuously for the first 2 hrs for any gross change in behavioral, neurologic and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 hrs and then again at 24, 48 and 72 hrs for any lethality or death. One-tenth of the maximum safe dose of the fractions and extract tested for acute toxicity was selected for the experiment [11].

**Multiple dose anti-hyperglycemic study**

Multiple dose study was conducted as per methods published previously [1,2,13]. The apportionment of animals into different groups were done as follows: Group I - Normal rats (No Treatment), Group II - Diabetic rats (treated with solvent 2 ml/kg), Group III - Diabetic rats (hydro-alcoholic extract 100 mg/kg) (HAARR 100 mg/kg), Group IV- Diabetic rats (treated with ethyl acetate fraction 100 mg/kg) (HAARE 100 mg/kg), Group V - Diabetic rats (treated with methanolic fraction 100 mg/kg) (HAARM 100 mg/kg), Group VI - Diabetic rats (treated with residual fraction 100 mg/kg) (HAARR 100 mg/kg), and Group VII - Diabetic rats (treated with metformin 300 mg/kg). The treatment was given continuously for 14 days and fasting blood samples were collected on day 0, 1, 3, 6, 9, and 14 for estimating blood sugar.

Physical parameters such as body weight, water, and food intake were observed on weekly basis whereas biochemical parameters such as serum creatinine, urea, triglyceride and total cholesterol were measured at the end of the study (day 15). Blood was collected for biochemical parameter observation after sacrificing the animals by decapitation. The biochemical parameters were measured using commercially available kits.

**Statistical analysis**

Results were expressed as a mean±standard error of the mean. The data were analyzed by one-way ANOVA followed by Turkey-Kramer multiple comparison test and p<0.05 was considered as significant.

**RESULTS**

**Effect of fractions and extract on blood glucose level of hyperglycemic rats**

Effect of the fractions on FBG level of hyperglycemic rats showed in Table 1. At the end of the 14 days treatment, percentage decrease in FBG level of hyperglycemic rats observed to be 3.67%, 14.03%, 47.69%, and 50.93% with respect to treatment with residual fraction, methanolic fraction, ethyl acetate fraction and standard drug, respectively, when compared with the level at start of the study (day 0). However, it can also be observed that the significant (p<0.001) reduction at the last day of observation found only in the groups treated with ethyl acetate fraction and standard drug when compared with diabetic control group. In other groups, no significant change was observed during the experimental period. The observations from a decrease in blood glucose level of hyperglycemic rats suggest that ethyl acetate fraction may be considered as a fraction with anti-hyperglycemic property and comparable with that of standard drug metformin.

**Effect on body weight**

The effect of the fractions against hyperglycemia induced loss of body weight of rats mentioned in Table 2. During the 1st week of observation (day 0 to day 7), decrease in mean body weight of the animals observed in all groups (except normal rats). The percentage decrease in mean body weight of animals in diabetic control group, extract treated group, residual fraction treated group, methanolic fraction treated group, ethyl acetate fraction treated group, and metformin-treated group during the 1st week of observation is 19.74%, 14.85%, 3.53%, 5.45%, 5.31%, and 20.27%, respectively. During this period, the animals treated with fractions had shown lesser percentage decrease in mean body weight and the mean body weight of animals in these groups is significantly different from the mean body weight of animals from the diabetic control group (p<0.001). No significant difference observed between the mean body weights of animals treated with different fractions during the 1st week. During the 2nd week of observation (day 7 to day 14) decrease in body weight of the animals observed only in groups treated either with solvent or extract or metformin. In all other groups, there is an increase in body weight with respect to the weight observed during the previous week. The percentage decrease in mean body weight of animals in diabetic control group, extract treated group, and metformin-treated group during 2nd week of observation is 16.37%, 18.88%, and 12.26%, respectively. The percentage increase in mean body weight of animals in groups treated with residual fraction, methanolic fraction, and ethyl acetate fraction is 1.92, 3.18 and 3.57%, respectively. The mean body weight of the animals in the groups treated with residual fraction, methanolic fraction and ethyl acetate fraction is significantly (p<0.001) different from the diabetic control group with no significant difference among themselves.

The observation of percentage change in mean body weight of animals of all groups during the study period (day 0 to day 14) revealed that decrease in mean body weight of animals in all groups (except normal rats) in range of 1.93-3.29%. The highest percentage decrease was in diabetic control group (32.88%) whereas the lowest decrease was in ethyl acetate fraction treated group (1.93). During the observation period, the animals treated with fractions had shown lesser percentage decrease in body weight and the mean body weight of animals in these groups is significantly (p<0.001) different from mean body weight of animals from the diabetic control group, with no significant difference among themselves. The percentage decrease in mean body weight of
Table 1: Effect of extract, fractions and standard drug on blood glucose level of hyperglycemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
<th>Day 13</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat</td>
<td>101.33±5.110</td>
<td>101.33±4.999</td>
<td>100.50±4.155</td>
<td>102.00±4.973</td>
<td>101.17±5.205</td>
<td>100.83±5.427</td>
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<tr>
<td>HAAR (100 mg/kg)</td>
<td>437.33±24.606</td>
<td>437.33±24.729</td>
<td>437.17±24.623</td>
<td>440.67±24.816</td>
<td>444.00±27.443</td>
<td>447.17±27.089</td>
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<tr>
<td>HAARR (100 mg/kg)</td>
<td>417.83±23.951</td>
<td>414.17±22.903</td>
<td>409.33±22.344</td>
<td>404.17±22.134</td>
<td>409.17±21.769</td>
<td>402.50±22.931</td>
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<tr>
<td>HAARM (100 mg/kg)</td>
<td>432.33±33.734</td>
<td>420.50±33.866</td>
<td>408.03±32.531</td>
<td>398.50±32.881</td>
<td>385.17±35.509</td>
<td>371.67±35.647</td>
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</tr>
<tr>
<td>HAAR (100 mg/kg)</td>
<td>447.67±32.597</td>
<td>407.50±35.085</td>
<td>372.83±35.25</td>
<td>358.67±36.53</td>
<td>326.33±31.41</td>
<td>234.17±22.846</td>
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</tr>
<tr>
<td>Metformin (300 mg/kg)</td>
<td>447.33±16.134</td>
<td>417.17±17.47</td>
<td>334.67±20.794</td>
<td>252.67±12.060</td>
<td>238.17±9.300</td>
<td>219.50±11.932</td>
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</tr>
</tbody>
</table>

Values expressed as mean±SEM; (n=6); One-way ANOVA followed by Turkey-Kramer multiple comparison test; *p<0.05, **p<0.01, ***p<0.001 versus diabetic control group comparison of blood glucose level done on the day 1; p<0.05, **p<0.01, ***p<0.001 versus diabetic control group comparison of body weight done on the day 7; p<0.05, **p<0.01, ***p<0.001 versus diabetic control group comparison of blood glucose level done on the day 14. STZ: Streptozotocin, HAAR: Hydro-alcoholic extract of A. reticulata, HAARR: Residual fraction of hydro-alcoholic extract of A. reticulata, HAAM: Methanolic fraction of hydro-alcoholic extract of A. reticulata, HAARE: Ethyl acetate fraction of hydro-alcoholic extract of A. reticulata, SEM: Standard error of the mean, A. reticulata: Annona reticulata

animals in extract treated and metformin-treated group is similar to that of diabetic control group.

**Effect on water intake and food intake of hyperglycemic rats**

The effects of the tests and standard substances on daily average water and food intake habit of hyperglycemic rats depicted in Tables 3 and 4, respectively. In all fraction treated groups the mean daily average water and food consumption lesser than that of the untreated diabetic animal group. In the case of daily average water intake observation; by end of week 2 the percentage decrease in the mean of daily average water intake of animals of the groups treated with methanolic fraction, ethyl acetate fraction, and metformin is 37.93, 50.43, and 49.79, respectively, and significantly (p<0.001) different from the value observed corresponding to diabetic control group. In case of residual fraction treated group, and metformin-treated group, respectively. In the case of total cholesterol level observation, the groups treated with residual fraction, ethyl acetate fraction, and metformin had shown significant (p<0.01-0.001) difference when compared with the diabetic control group, whereas the methanolic fraction treated group and extract treated group did not show any significant difference with the diabetic control group. The cholesterol levels of different groups at end of the experimental duration of 14 days were 318.973, 316.843, 116.20, 195.49, 198.32, and 97.33 mg/dL corresponding to diabetic control group, extract treated group, residual fraction treated group, and metformin-treated group, respectively.

**Effect on observed Biochemical parameters**

The effect of all fractions on biochemical parameters such as creatinine, urea, cholesterol, and triglyceride presented in Table 5. From the results it’s evident that, effects of all the fractions on the observed biochemical parameters are more or less similar. By the end of week 2 in all fraction and standard treated groups the triglyceride level found to be significantly (p<0.001) lesser than the diabetic control group. The triglyceride levels of different groups at end of the experimental duration of 14 days were 318.973, 316.843, 116.20, 195.49, 198.32, and 97.33 mg/dL corresponding to diabetic control group, extract treated group, and metformin treated group, respectively. In the case of creatinine level residual fraction, methanolic fraction and metformin had shown significant (p<0.01-0.001) difference when compared with the diabetic control group. Treatment with extract or ethyl acetate fraction did not bring any change in the creatinine level to be significantly different when compared with the creatinine level of the diabetic control group. In the case of urea only ethyl acetate fraction treated group has shown significant (p<0.01) decrease when compared with the diabetic control group. The metformin-treated group had also shown significant (p<0.001) difference with the diabetic control group when the urea level is compared on day 14, but the value is higher than that of the diabetic control group.

**DISCUSSION**

The current study focuses on the efficiency of different fractions prepared from hydro-alcoholic extract of leave of A. reticulata in the regulation of blood glucose levels in STZ induce hyperglycemic rats.
Table 3: Effect of test extract, fractions, and standard on average water intake habit of hyperglycemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average water intake rat (ml/rat/day) During W0</th>
<th>During W1</th>
<th>During W2</th>
<th>% change between week 0 and week 1</th>
<th>% change between week 1 and week 2</th>
<th>% change between week 0 and week 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat</td>
<td>32.33±1.706</td>
<td>32.17±0.5426</td>
<td>31.67±1.542</td>
<td>-0.49</td>
<td>-1.55</td>
<td>-2.04</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>76.83±6.009</td>
<td>89.67±1.085</td>
<td>100.17±1.662</td>
<td>16.71</td>
<td>11.71</td>
<td>30.38</td>
</tr>
<tr>
<td>HAAR (100 mg/kg)</td>
<td>79.67±2.552</td>
<td>90.17±2.428</td>
<td>100.50±2.742</td>
<td>13.18</td>
<td>11.46</td>
<td>26.15</td>
</tr>
<tr>
<td>HAARR (100 mg/kg)</td>
<td>78.50±1.258</td>
<td>90.50±1.544</td>
<td>99.50±1.688</td>
<td>15.29</td>
<td>9.94</td>
<td>26.75</td>
</tr>
<tr>
<td>HAARM (100 mg/kg)</td>
<td>78.67±6.667</td>
<td>66.67±2.092***</td>
<td>48.83±2.301***</td>
<td>-15.25</td>
<td>-26.67</td>
<td>-37.93</td>
</tr>
<tr>
<td>HAARE (100 mg/kg)</td>
<td>78.33±1.926</td>
<td>65.50±2.277***</td>
<td>38.83±0.654***</td>
<td>-16.38</td>
<td>-40.72</td>
<td>-50.43</td>
</tr>
<tr>
<td>Metformin in 300 mg/kg</td>
<td>79.33±0.9545</td>
<td>59.17±1.041***</td>
<td>39.83±1.302***</td>
<td>-25.41</td>
<td>-32.69</td>
<td>-49.79</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM; (n=6); One-way ANOVA followed by Turkey-Kramer multiple comparison test; *p<0.05, **p<0.01, ***p<0.001 versus diabetic control group at end of week 2. HAAR: Hydro-alcoholic extract of A. reticulata, HAARR: Residual fraction of hydro-alcoholic extract of A. reticulata, HAARM: Methanolic fraction of hydro-alcoholic extract of A. reticulata, HAARE: Ethyl acetate fraction of hydro-alcoholic extract of A. reticulata, SEM: Standard error of the mean, A. reticulata: Annona reticulata

Table 4: Effect of test extract, fractions, and standard on average food intake habit of hyperglycemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average daily food intake (g/rat/day) During W0</th>
<th>During W1</th>
<th>During W2</th>
<th>% change between week 0 and week 1</th>
<th>% change between week 1 and week 2</th>
<th>% change between week 0 and week 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat</td>
<td>13.18</td>
<td>-15.25</td>
<td>89.177±6.219</td>
<td>31.67±1.542</td>
<td>10.88</td>
<td>53.59</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>26.17±0.4944</td>
<td>21.17±0.5426</td>
<td>21.17±0.5426</td>
<td>3.21</td>
<td>-4.51</td>
<td>-2.31</td>
</tr>
<tr>
<td>HAAR (100 mg/kg)</td>
<td>26.67±7.6701</td>
<td>37.67±7.102</td>
<td>41.33±0.954</td>
<td>21.42</td>
<td>9.72</td>
<td>54.97</td>
</tr>
<tr>
<td>HAARR (100 mg/kg)</td>
<td>29.17±0.4773</td>
<td>36.17±1.138</td>
<td>36.83±1.167***</td>
<td>23.99</td>
<td>1.82</td>
<td>26.26</td>
</tr>
<tr>
<td>HAARM (100 mg/kg)</td>
<td>28.67±7.149</td>
<td>24.50±0.8466***</td>
<td>21.17±0.609***</td>
<td>-14.54</td>
<td>-13.59</td>
<td>-26.16</td>
</tr>
<tr>
<td>HAARE (100 mg/kg)</td>
<td>29.17±0.6009</td>
<td>23.67±0.819***</td>
<td>19.33±0.760***</td>
<td>-18.86</td>
<td>-18.34</td>
<td>-33.73</td>
</tr>
<tr>
<td>Metformin in 300 mg/kg</td>
<td>28.17±0.4737</td>
<td>19.17±0.8724***</td>
<td>19.17±0.609***</td>
<td>-31.95</td>
<td>0.90</td>
<td>-31.95</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM; (n=6); One-way ANOVA followed by Turkey-Kramer multiple comparison test; *p<0.05, **p<0.01, ***p<0.001 versus diabetic control group at end of week 1; *p<0.05, **p<0.01, ***p<0.001 versus diabetic control group at end of week 2. HAAR: Hydro-alcoholic extract of A. reticulata, HAARR: Residual fraction of hydro-alcoholic extract of A. reticulata, HAARM: Methanolic fraction of hydro-alcoholic extract of A. reticulata, HAARE: Ethyl acetate fraction of hydro-alcoholic extract of A. reticulata, SEM: Standard error of the mean, A. reticulata: Annona reticulata

Table 5: Effect of test extract, fractions, and standard drug on biochemical parameters of hyperglycemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urea (mg/dl) During W0</th>
<th>Creatinine (mg/dl) During W0</th>
<th>Cholesterol (mg/dl) During W0</th>
<th>Triglyceride (mg/dl) During W0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat</td>
<td>27.47±2.879</td>
<td>0.545±0.0376</td>
<td>49.25±5.496</td>
<td>89.177±26.19</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>64.59±2.577</td>
<td>1.085±0.0762</td>
<td>138.48±13.426</td>
<td>318.973±24.561</td>
</tr>
<tr>
<td>HAAR (100 mg/kg)</td>
<td>62.49±2.523</td>
<td>0.958±0.0490</td>
<td>135.47±12.614</td>
<td>316.84±23.343</td>
</tr>
<tr>
<td>HAARR (100 mg/kg)</td>
<td>60.46±2.377</td>
<td>0.727±0.0491***</td>
<td>78.90±10.229**</td>
<td>116.20±5.342***</td>
</tr>
<tr>
<td>HAARM (100 mg/kg)</td>
<td>46.40±2.345**</td>
<td>0.657±0.0619***</td>
<td>114.45±5.531</td>
<td>195.49±10.038**</td>
</tr>
<tr>
<td>HAARE (100 mg/kg)</td>
<td>39.72±2.877**</td>
<td>0.915±0.0664***</td>
<td>61.65±5.255**</td>
<td>198.32±15.827**</td>
</tr>
<tr>
<td>Metformin in 300 mg/kg</td>
<td>104.97±5.838***</td>
<td>0.638±0.0367***</td>
<td>33.59±1.550***</td>
<td>97.33±10.330***</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM; (n=6); One-way ANOVA followed by Turkey-Kramer multiple comparison test; *p<0.05, **p<0.01, ***p<0.001 versus diabetic control group. HAAR: Hydro-alcoholic extract of A. reticulata, HAARR: Residual fraction of hydro-alcoholic extract of A. reticulata, HAARM: Methanolic fraction of hydro-alcoholic extract of A. reticulata, HAARE: Ethyl acetate fraction of hydro-alcoholic extract of A. reticulata, SEM: Standard error of the mean, A. reticulata: Annona reticulata

Table 1 represents the effect of the fractions on blood glucose level of STZ-induced diabetic rats. From the results, it is evident that only ethyl acetate fraction is capable to reduce the blood glucose level significantly (p<0.001) at the tested dose level, whereas the other fractions did not show any significant glucose lowering activity. Hence, the ethyl acetate fraction may be considered as the fraction having potential anti-hyperglycemic activity. The results observed may be attributed to the compounds present in ethyl acetate fraction. The major compounds 9-octadecenamide, (Z)-, ethyl isoallocholate and 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol, (3α,5Z,7E)- present in ethyl acetate fraction [8] may be considered as compounds responsible for the observed anti-hyperglycemic activity. The compound 9-octadecenamide, (Z)- and it’s derivatives are known to be potent hypolipidemic agent. They have effect on pepsinogen prohormone-activated receptor-α, tumor necrosis factor α, and potential antioxidant effect as well [18-20]. The presence of these compounds may be considered as responsible for the observed anti-hyperglycemic effect of the ethyl acetate fraction. The observation of chemical components of methanolic and residual fraction revealed glicyrine as the major constituent [8]. Glicyrine is capable of causing hyperglycemia in animals after oral or intraperitoneal administration [21]. The phytochemical analysis also revealed that methanolic fraction and residual fraction contains several other compounds such as D-glucose, D-α-D-galactopyranosyl-, α-D-glucopyranoside, α-D-glucopyranosyl-(1→2)α-D-fructofuranosyl, α-D-Glucopyranose, 4-O-α-D-Galactopyranosyl-, α-methyl-D-Mannopyranoside which are
sugar like [8] in nature. Hence, the presence of glycerine and other sugar like compounds as major components may be the reason due to which the methanolic fraction and residual fraction had not been able to show any kind of anti-hyperglycemic effect at the tested dose level. Natural compounds like flavonoids and phenolics are known to have antioxidant property and prevent free radical chain reaction in biological systems and provide additional health benefits [22]. Flavonoids are known as excellent free radical scavenging agents and known to have properties for the management of diabetes and diabetic complications [23-25]. Flavonoids also lead to regeneration of pancreatic β-cells, reduce necrosis and degeneration of pancreatic β-cells and thus may be effective in treating hyperglycemia by preventing diabetic complications [26]. Flavonoids are also known to decrease triglyceride level [27]. Phenolic compounds are also known to decrease blood glucose in STZ-induced diabetic rats and increase in insulin secretion, which may be due to regeneration of β-cells brings by reduction in oxidative stress [28]. Phenolics tend to increase the glutathione levels and thus decreases levels of lipid peroxidation in diabetic rats, and contribute in the management of diabetes and diabetic complications [29]. Ethyl acetate fraction had been found to have a high content of flavonoids and phenolics in comparison to other tested fractions [30] and hence may be another reason for the observed anti-hyperglycemic potential.

Sreptozotocin induced hyperglycemia is associated with characteristic loss of body weight because of increased muscle wasting and loss of tissue proteins [31]. During the observation of the effect of the fractions on body weight loss, it is observed that all the fractions at the tested dose level are capable of arresting the loss of body weight significantly. Oxidative stress produced due to STZ administration is known to impair muscle repair and antioxidants are capable of improving oxidative imbalance and improve muscle repair [32]. The tested fractions had been identified to have antioxidant potential [30] and hence may be one of the reasons for prevention of loss of body weight by the fractions. Increase in body weight or prevention of loss of body weight in ethyl acetate fraction administered group may also be attributed to the components of the fraction which are capable of attenuating hyperglycemia condition. In the case of methanolic fraction and residual fraction administered group, the increase in body weight may also be due to the presence of sugar like compounds [33]. The extract at the tested dose level (100 mg/kg) did not show any protective effective against body weight loss. The extract had exhibited an increase in body weight of STZ induced hyperglycemic rats when administered at a dose level of 200-400 mg/kg [7], hence the diminished effect of the extract may be attributed to the decreased dose level. The standard drug metformin did not provide any protection to body weight loss. Metformin is known to reduce body weight due to decreased calorie intake [34] and hence the observation is in line with the property of the standard drug.

Sreptozotocin induced hyperglycemia is always accompanied by polydipsia and polyphagia [35]. In this study, significant (p<0.001) decrease in water intake can be observed in the groups treated with methanolic fraction, ethyl acetate fraction, and metformin in comparison to diabetic control group. In the case of food intake habit, the groups treated with all fractions and metformin had shown significant (p<0.001) decrease in food intake as compared to the diabetic control group. The significant decrease in water and food intake in case of ethyl acetate treated group may be due to the observed anti-hyperglycemic activity of the drug substance. Hence, the positive effect of ethyl acetate fraction on the secondary parameters like food and water intake supports the anti-hyperglycemic of the fraction.

Dyslipidemia is known as one of the major risk factors associated with diabetes, characterized by increased plasma triglyceride level, decreased high-density lipoprotein (HDL) cholesterol level, and increased low-density lipoprotein (LDL)-cholesterol level. The changes in lipid profile of diabetic patients are associated with increased free fatty acid flux and secondary to insulin resistance [36,37]. Standard drug metformin is known to have a beneficial effect in decreasing the level of triglycerides and LDL-cholesterol by 10-15% along with slight increase in HDL levels [38]. Hence, the observed effect of metformin on lipid profile of diabetic rats is in agreement with the property of the drug. As the results suggest ethyl acetate fraction is capable to control the level of triglyceride and cholesterol significantly (p<0.001) in hyperglycemic rats. The residual fraction is also been able to reduce the cholesterol (p<0.001) and triglyceride (p<0.01) level significantly when compared with the diabetic control group. The treatment with methanolic fraction is capable to reduce only triglyceride level significantly (p<0.001) with no significant effect on the cholesterol level. Oxidative stress has been shown to play a role in the causation of diabetes [39]. STZ produces oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood sugar seen in animals [40]. Increase in systemic oxidative stress is considered as one of the factors that may contribute to the development of obesity, atherosclerosis, and other cardiovascular risk associated with which are associated with diabetes and another metabolic syndrome [41,42]. The fractions used in the current experiment also had potential antioxidant activity [30] and may be one of the reasons for controlling cholesterol and/or triglyceride level of diabetic rats. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridermia and hypercholesterolemia [43]. The ethyl acetate fraction has shown significant anti-hyperglycemic potential and hence increase insulin secretion and/or insulin sensitization may be proposed to be another reason for the control of hypertriglyceridermia and hypercholesterolemia by the ethyl acetate fraction.

Increase in level of urea and creatinine is indicators of impaired renal function [44] and may not be seen all diabetic patients [45]. Induction of experimental diabetes by injecting STZ may alter kidney morphology and kidney function leading to diabetic nephropathy [46,47]. In this study, increase in the level of creatinine and urea was observed in all groups of diabetic rats and remained elevated in comparison with the normal rats till end of the experimental period. The treatment with residual fraction, methanolic fraction, and metformin has decreased the level of creatinine significantly (p<0.001) when compared with the diabetic control group. Ethyl acetate fraction had not shown any kind of controlling effect on increased creatinine level. The urea level is controlled significantly (p<0.001) only in ethyl acetate fraction treated group in comparison to diabetic control group. In metformin-treated group, there was a significant (p<0.001) increase in urea level. In STZ induced diabetic models oxidative stress and reactive oxygen species play major roles in glucose-induced renal injury [48-51]. The effect of the fractions on the renal function of diabetic rats had been partial in providing protection either against increased creatinine level and/or against urea level and may be attributed to the antioxidant property of the fractions [30]. Effect of metformin in diabetic nephropathy is not clear and it is contraindicated in case of renal impairment and should not be used until renal function is normal [43]. However, some studies suggest that metformin may have some renoprotective activity [52]. Metformin is suggested to provide protection against STZ induced nephropathy by restoration of biochemical alterations and modulation of oxidative stress [53]. Hence, the effect of metformin on decreasing creatinine level may be due to restoration of biochemical alterations and/or modulation of oxidative stress. Metformin is known to be associated with a very rare, preventable but fatal adverse effect lactic acidosis [43], which may be associated with increase in urea level [54,55]. Hence, the increase in urea level may be due to metformin-induced lactic acidosis.

From the results of the study, it is evident that among the tested fractions ethyl acetate fraction is having better anti-hyperglycemic potential. Studies suggest that that the extracts of leaves of A. reticulata have the potential to inhibit the action of α-amylase and α-glucosidase [56]. Thus, the observed anti-hyperglycemic activity of ethyl acetate
fraction may be attributed to the antioxidant property of the fraction and/or to inhibition of action of α-amylase and/or α-glucosidase and/or insulin secretion and/or insulin sensitization. The observed anti-hyperglycemic effect of the fraction may be due to one or more compounds present in the fraction. Further experimental observations are warranted to understand the mechanism/process involved.

CONCLUSION

Ethyl acetate fraction prepared from hydro-alcoholic extract of A. reticulata leaf is having anti-hyperglycemic potential and the observed glucose lowering activity may be due to the presence of one or more pharmacologically active compounds in the fraction. Further studies are recommended to comprehend the exact mechanism of action and the compounds responsible for the action.

ACKNOWLEDGMENT

The authors are grateful to the president and vice-chancellor, S ‘O’ A University, Bhubaneswar, Odisha, India for providing necessary facilities to carry out the research work in the faculty of pharmacy, S ‘O’ A University.

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